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Glucocorticoid Regulation of Hepatic *S*-Adenosylmethionine Synthetase Gene Expression*

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ABSTRACT

The effects of glucocorticoids on the regulation of rat liver *S*-adenosylmethionine synthetase were studied *in vivo* and in two culture systems. Livers from adrenalectomized animals were examined for enzyme activity, immunoreactive protein, and messenger RNA (mRNA) content. All three parameters showed a similar trend, *i.e.* they decreased 3-fold after adrenalectomy and increased over the control values upon triamcinolone replacement. These results suggested that glucocorticoid regulation of hepatic *S*-adenosylmethionine synthetase was mediated at the mRNA level. Triamcinolone and dexamethasone increased *S*-adenosylmethionine synthetase mRNA content in a time- and dose-dependent manner in both rat hepatoma H35 cells and primary cultures of adult rat hepatocytes. The kinetics of mRNA induction were identical in both culture systems, indicating that the hormone-mediated response is independent of the differentiated state of the cell. Insulin blocked the inducing effect of glucocorticoids on *S*-adenosylmethionine synthetase mRNA in a dose-dependent manner. On the other hand, the triamcinolone-dependent increase in mRNA levels was completely abolished by treatment with actinomycin D, whereas cycloheximide did not affect this response. The transcription rate of the gene, as measured by run-on assay, increased 3-fold after hormone addition. Transient transfections of H35 cells with 1.4 kilobases of the 5′-flanking region of the hepatic *S*-adenosylmethionine synthetase gene fused to a luciferase reporter gene showed that promoter activity is also increased 3-fold after triamcinolone treatment, suggesting that this promoter region contains the sequence elements necessary to confer glucocorticoid responsiveness. In addition to the transcriptional control of the hepatic *S*-adenosylmethionine synthetase gene, our results suggest that glucocorticoids may be acting at a posttranscriptional level. (*Endocrinology* **138:** 1251–1258, 1997)

S-ADENOSYLMETHIONINE (AdoMet) synthetase (EC 2.5.1.6) is a housekeeping enzyme that catalyzes the only known route of biosynthesis of AdoMet in a reaction that involves the transfer of the adenosyl moiety from ATP to the sulfur atom of methionine (1). The relevance of this reaction has been recently emphasized by the fact that AdoMet synthetase has been found to be included in the minimal set of functional gene products necessary for independent life (2). This is due to the central position that AdoMet occupies in cellular metabolism; it is the donor of methyl groups for most of transmethylation reactions, which are essential to maintain cellular structure and function, and serves as the source of propylamine moieties for polyamine biosynthesis (3, 4). In mammalian liver, this metabolite also participates in the transsulfuration pathway (5), which leads to the synthesis of compounds involved in maintaining the normal redox potential, such as glutathione. Among mammalian tissues, liver exhibits the highest enzyme specific activity, consistent with the fact that the majority of methionine taken up from the diet is metabolized by this pathway in this organ (6). Related to this, an AdoMet synthetase isoenzyme is selectively expressed in the liver, whereas in other tissues, the so-called extrahepatic or kidney-type AdoMet synthetase is present (reviewed in Refs. 7 and 8).

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Over the past few years, much effort has been devoted to study of the regulation of hepatic AdoMet synthetase under various pathophysiological conditions. The specific activity of the enzyme has been shown to be altered in response to tissue damage, concomitantly with a severe impairment of methionine metabolism. For instance, a marked reduction in enzyme activity has been found in human alcoholic (9, 10), biliary (11), and posthepatitic cirrhosis (9) as well as in different experimental models of liver injury (see Ref. 8 and references therein). Various pieces of evidence have led to the suggestion that the activity of the enzyme could be regulated under these conditions by the oxidation state of its thiol groups (12–15). The involvement of other possible regulatory mechanism, such as the modulation of the messenger RNA (mRNA) levels, has been also explored. Thus, in a rat model of carbon tetrachloride-induced liver damage, despite the marked reduction in enzyme activity, the levels of the corresponding mRNA have been found to be similar to those detected in normal liver (16), a finding which agrees with that derived from the analysis of liver biopsies from alcoholic cirrhotic patients (17). These results supported the idea that the decrease in hepatic AdoMet synthetase activity observed under several pathological situations was due to posttranslational events rather than to a reduced expression of the gene.

The activity of the enzyme has been also found to be influenced by hormonal stimuli. In this regard, it was previously reported that hepatic AdoMet synthetase activity is altered in adrenalectomized animals (18–20), suggesting a role for glucocorticoids in its regulation. These hormones account for pleitropic effects in liver, and its action on hepatic AdoMet synthetase could be of major physiological rele-

vance. However, the mechanisms underlying this regulation remain unknown to date. The present work was undertaken to investigate such mechanisms. Our results show that glucocorticoids strongly up-regulate this enzyme both *in vivo* and in hepatic cultured cells and have a direct effect on enzyme gene expression. The physiological significance of this regulation is further discussed.

Materials and Methods

Animals and in vivo procedures

Male Wistar rats, weighing 180–210 g, were maintained in temperature-controlled rooms under 12-h light-dark cycles, with standard diet provided *ad libitum*. Bilateral adrenalectomy or sham operation was performed under ether anesthesia. Adrenalectomized animals received 0.9% NaCl in their drinking water. Triamcinolone (Sigma Chemical Co., St. Louis, MO; 5 mg/100 g BW) was injected ip at two consecutive 24-h intervals, 7 days after adrenalectomy. Livers were freeze-clamped in liquid nitrogen and stored at −70 C before the extraction of proteins and mRNA. All animal procedures were approved by the animal care and use committee of our institution.

Isolation and culture of rat hepatocytes

Hepatocytes were isolated by the collagenase perfusion method (21) with the modifications previously outlined (22). Only cell preparations whose viability, estimated by the trypan blue exclusion test, was higher than 90% were used. Hepatocytes were seeded at a density of 5×10^4 cells/cm^2 in RPMI supplemented with 10% FCS, 2 mM glutamine, and antibiotics at 37 C in an humidified atmosphere of 5% CO_2-95% air. After a 4-h incubation period, the medium was replaced by serum-free medium and incubated for 16 h. Then, treatments with triamcinolone, dexamethasone, or vehicle (methanol) at the indicated concentrations were started.

Cell culture

Rat hepatoma H35 cells were grown to 70–80% confluence in DMEM supplemented with 10% FCS. The cells were changed to a medium containing 0.5% FCS 24 h before the addition of hormones or vehicle. For time-course experiments, cells were harvested at the indicated times after the addition of 100 nM triamcinolone or dexamethasone. Triamcinolone and dexamethasone were dissolved in methanol and ethanol, respectively. When required, actinomycin D and cycloheximide (Sigma) were used at concentrations of 5 and 10 μg/ml, respectively. Human insulin (Novo Nordisk, Copenhagen, Denmark) was used at the indicated concentrations.

Determination of AdoMet synthetase activity

For determination of AdoMet synthetase activity, liver portions were homogenized at 4 C in 4 vol 10 mM Tris-HCl pH 7.5, containing 0.3 M sucrose, 0.1% β-mercaptoethanol, 1 mM benzamidine, and 0.1 mM phenylmethylsulfonylfluoride. The homogenate was centrifuged at 4 C for 20 min at 10,000 × *g*, and the supernatant was again centrifuged for 60 min at 100,000 × *g*. AdoMet synthetase activity was assayed in this last supernatant as previously described (23). Samples of 160 μl were incubated with 90 μl of a reaction mixture containing 75 mM Tris-HCl (pH 8.0), 250 mM KCl, 9 mM $MgCl_2$, 10 mM dithiothreitol, 5 mM methionine, and 5 mM [2-^{3}H]ATP (4Ci/mol). The incubation was carried out for 30 min at 37 C and was stopped by the addition of 4–5 ml distilled water. The samples were immediately loaded on 1-ml cation exchanger AG 50W-X4 columns (Bio-Rad Laboratories, Richmond, CA) equilibrated in water. The columns were washed with water (20 ml), and [^{3}H]AdoMet was then eluted with 4 ml 3 M ammonium hydroxide. Radioactivity was determined in the presence of 1 ml glacial acetic acid and 10 ml scintillation liquid (Optiphase HiSafe 3, Pharmacia, Uppsala, Sweden).

Northern blot analysis

Total RNA was isolated by the guanidinium isothiocyanate method (24), size-fractionated on 1% agarose denaturing gels, and transferred to Nytran membranes (Schleicher and Schuell, Keene, NH). The filters were hybridized with an *Eco*RI fragment of plasmid pSSRL, containing 2.2 kilobases (kb) of rat liver AdoMet synthetase complementary DNA (cDNA) (25). A cDNA for the 18S ribosomal RNA inserted into plasmid pBR322 and/or a rat glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA (26) (provided by Dr. R. Wu) were used for normalization. The probes were random primed labeled with [α-^{32}P]deoxy-CTP using the Megaprime labeling kit (Amersham, Little Chalfont, UK). Prehybridization and hybridization were carried out as previously described (27). The filters were scanned on a Molecular Imager GS-250 (Bio-Rad Laboratories, Richmond, CA), and quantitative analysis of the autoradiograms was carried out running the Phosphor analyst software (Bio-Rad Laboratories, Richmond, CA).

Western blot analysis

Samples from rat liver or H35 cells were homogenized in 10 mM Tris-HCl, pH 7.5–0.3 M sucrose buffer, as indicated, for determination of AdoMet synthetase activity. Forty micrograms of proteins from the cytosolic fractions were fractionated by 10% SDS-PAGE gels and electrotransferred to nitrocellulose membranes using 20 mM Tris-HCl pH 7.5, containing 20% methanol. After transfer, the blots were incubated consecutively with a rabbit antiserum raised against purified rat liver-specific AdoMet synthetase (28) (dilution, 1:10000) and horseradish peroxidase-conjugated antirabbit IgG. Blots were developed with ECL detection reagents (DuPont-New England Nuclear, Boston, MA), following the manufacturer's instructions. The immunoblot bands were measured by densitometric analysis of the autoradiographs. The amount of the sample used and the time of exposure to the x-ray film were chosen to give a linear response.

Nuclear run-on analysis

Nuclei from H35 cells untreated or treated for 1 and 6 h with 100 nM triamcinolone were isolated as previously described (29). For each preparation of nuclei, three 10-cm dishes (1.5×10^7 cells) were used. Nuclear suspensions (100 μl) were incubated with 0.5 mM each of CTP, ATP, and GTP and with 200 μCi [α-^{32}P]UTP (3000 Ci/mmol; DuPont-New England Nuclear). The ^{32}P-labeled RNA was isolated after deoxyribonuclease I and proteinase K treatments by phenol-chloroform extraction and ethanol precipitation; then it was further purified by Sephadex G-50 chromatography. Equal amounts (10^7 cpm/ml) of labeled nuclear RNA were hybridized at 42 C for 72 h to 5 μg linearized plasmid DNAs immobilized on Nytran membranes. The plasmids used were pSSRL (25), plasmid pPCK10 (30) (provided by Dr. R. W. Hanson), containing a 2.6-kb cDNA for rat cytosolic phosphoenolpyruvate carboxykinase (PEPCK) cDNA, a rat GAPDH cDNA (26), and pUC18 (for background control). After washing, the filters were exposed to a phosphorimaging screen (Bio-Rad) for quantification of the radioactive transcripts. Data were normalized to transcription of the GAPDH gene.

Transient transfection analysis

A rat genomic DNA fragment containing 1.4 kb of a recently cloned rat liver-specific AdoMet synthetase promoter (L. Alvarez, E. Sánchez-Góngora, J. Mingorance, B. Gil, M. A. Pajares, and J.M. Mato, manuscript in preparation) was used to drive expression of the luciferase gene in the promoterless vector pXP1 (31) (kindly provided by Dr. S. K. Nordeen). The β-galactosidase expression vector pCH110 (Pharmacia) was used as an internal standard of transfection efficiency. H35 cells were cultured in DMEM supplemented with 10% FCS and plated at approximately 3×10^5 cells/60-mm culture dish 24 h before transfections. The cells were transfected by the calcium phosphate precipitation method (32) with either 15 μg of the AdoMet synthetase promoter-luciferase construct or pXP1 vector and 5 μg of the pCH110 plasmid. After 18 h, the DNA precipitates were rinsed twice with PBS, and cells were incubated in DMEM supplemented with 0.5% FCS and treated for 24 h with or without 100 nM triamcinolone. The cells were harvested in reporter lysis buffer (Promega, Madison, WI), following the manufacturer's instructions and the lysate was spun in a microcentrifuge for 15 sec. Luciferase and β-galactosidase activities were determined as previously described (32, 33). All transfections were conducted in triplicate, using at least two

different batches of each plasmid. The activities reported are averaged from three independent experiments.

Results

Effects of adrenalectomy and triamcinolone replacement on hepatic AdoMet synthetase activity, immunoreactive protein, and mRNA levels

The regulation of hepatic AdoMet synthetase by glucocorticoids was first examined *in vivo* in adrenalectomized rats. In liver from adrenalectomized animals, the specific activity of the enzyme, assayed at saturating concentrations of the substrates, ATP and methionine, was determined to be 48 ± 8 pmol/min·mg protein, significantly lower than that obtained in liver from sham-operated rats (158 ± 12 pmol/min·mg protein; Fig. 1A). Administration of the synthetic glucocorticoid triamcinolone (5 mg/100 g BW; two consecutive doses at daily intervals) to adrenalectomized rats restored AdoMet synthetase activity, which reached a value of 196 ± 15 pmol/min·mg protein. To investigate whether these changes in enzyme activity were associated with relative changes in the amount of the corresponding protein, the same cytosolic extracts used for determination of enzyme activity were subjected to immunoblotting. As shown in Fig. 1B, a marked reduction in immunoreactive protein was observed in liver extracts from adrenalectomized rats compared to that in liver extracts from sham-operated controls. Triamcinolone replacement increased the levels of protein over the values detected in the sham group. Thus, changes in AdoMet synthetase activity with adrenalectomy and triamcinolone replacement appear to reflect changes in the corresponding protein. To define further the molecular basis for AdoMet synthetase regulation, total RNA was isolated and subjected to Northern blot analysis. The abundance of the AdoMet synthetase mRNA changed in a similar fashion as the protein. Thus, a 3-fold reduction in hepatic AdoMet synthetase mRNA levels was detected in adrenalectomized rats compared with sham-operated controls; again, this effect was prevented by hormone treatment (Fig. 1C). Adrenalectomy and triamcinolone replacement had no effect on the levels of 18S ribosomal RNA (Fig. 1C) or GAPDH mRNA (data not shown). Overall, there was a strong correlation between the activity values and protein and mRNA contents, suggesting an *in vivo* regulatory mechanism acting primarily at the mRNA level.

Up-regulation of hepatic AdoMet synthetase by glucocorticoids in rat hepatoma H35 cells

To specifically study the effect of glucocorticoids on the regulation of this enzyme, two culture systems, namely rat hepatoma H35 cells and primary cultures of adult rat hepatocytes, were used. Initially, H35 cells were tested for AdoMet synthetase mRNA expression after stimulation with triamcinolone. As shown in Fig. 2A, triamcinolone significantly up-regulated AdoMet synthetase mRNA expression

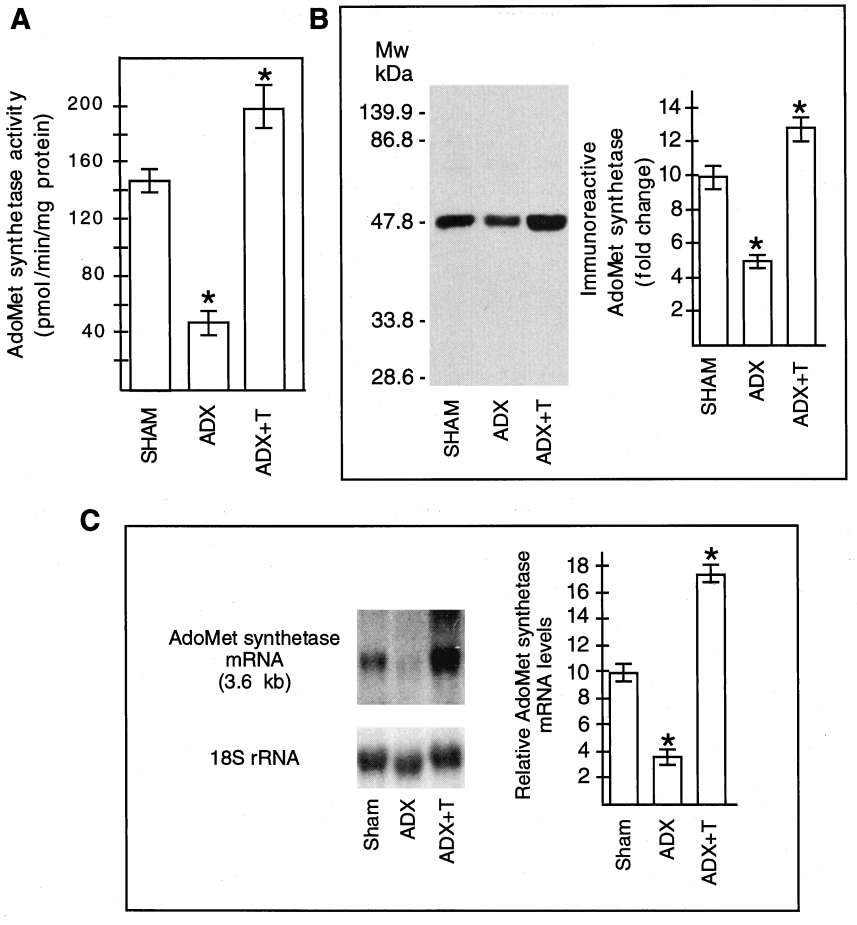

FIG. 1. Effect of adrenalectomy and triamcinolone replacement on rat liver AdoMet synthetase activity, protein, and mRNA levels. Sham-operated or adrenalectomized (ADX) animals were either injected with 0.9% saline or treated with triamcinolone (50 mg/kg, ip) for 48 h. A, AdoMet synthetase activity, assayed at saturating concentrations of the substrates, as described under *Materials and Methods*. B, Western blot analysis. Equal amounts of cytosolic proteins (30 μg) from the same samples used for determination of enzyme activity were fractionated by 10% SDS-PAGE and subjected to immunoblot analysis with an antibody raised against rat liver AdoMet synthetase (dilution, 1:10,000). C, Northern blot analysis, performed with 30 μg total RNA. Filters were hybridized to a ^{32}P-labeled rat liver AdoMet synthetase cDNA or to a rat 18S ribosomal RNA probe. Corrected densitometric quantitation is shown. Values are expressed as the mean ± SEM of four animals in each of the groups. *, $P < 0.01$ *vs.* sham group.

in a time-dependent manner. The mRNA levels of hepatic AdoMet synthetase detected in this cell line were low in the absence of hormone, but began to increase 2 h after the addition of triamcinolone and reached a maximum at 24 h (14-fold induction), decreasing slightly at 48 h. The levels of immunoreactive protein followed a trend similar to mRNA expression (Fig. 2B), but a delay was detected, reaching a plateau at 36 h (Fig. 2C). Identical results were obtained when another synthetic glucocorticoid, dexamethasone, was used (data not shown). Thus, as observed in adrenalectomized animals, the expression profile of immunoreactive protein paralleled that for the mRNA, further indicating that glucocorticoids modulate AdoMet synthetase expression by acting mainly at the mRNA level. Therefore, subsequent experiments were focused on mRNA expression.

The triamcinolone-mediated induction of AdoMet synthetase mRNA was dose dependent. As shown in Fig. 3, AdoMet synthetase mRNA expression gradually increased with the triamcinolone concentration, yielding a sigmoidal curve with a half-maximal mRNA induction at a concentration between 10^{-9}–10^{-8} M. The levels of corticosterone, which is the natural glucocorticoid in rat and is an order of magnitude less potent than synthetic glucocorticoids, show a daily rhythm, ranging from 10^{-7}–10^{-6} M (34). Therefore, it can be concluded that glucocorticoids control the expression of the hepatic AdoMet synthetase gene in their physiological concentration range.

Effects of triamcinolone on hepatic AdoMet synthetase mRNA in primary cultures of adult rat hepatocytes

The regulation of AdoMet synthetase gene expression was also examined in primary cultures of hepatocytes, because they resemble the normal functions of liver cells *in vivo* more closely than hepatoma cells. In this case, the steady state levels of hepatic AdoMet synthetase mRNA detected were much higher than those in the rat hepatoma cell line. Regardless of this, triamcinolone increased mRNA content in a time- and dose-dependent manner to a similar extent as observed in H35 cells (Fig. 4).

Glucocorticoid-insulin antagonism in the regulation of AdoMet synthetase mRNA

Previous studies have shown that hepatic AdoMet synthetase activity is increased in alloxan diabetic rats (19, 35, 36). Based on this and the fact that the actions of glucocorticoids in the regulation of various metabolic pathways are opposed by insulin, we next examined whether insulin was able to antagonize the stimulatory effect of triamcinolone on AdoMet synthetase mRNA expression. For this purpose, insulin at different concentrations was added to rat hepatoma H35 cells simultaneously with a fixed dose of triamcinolone (100 nM), which by itself caused a maximal induction of the mRNA. Under these conditions, glucocorticoid stimulation was inhibited about 50% by an insulin concentration of 10^{-8} M and was totally suppressed by 10^{-6} M (Fig. 5). In parallel experiments, insulin alone had negligible effects on hepatic AdoMet synthetase mRNA expression (data not shown).

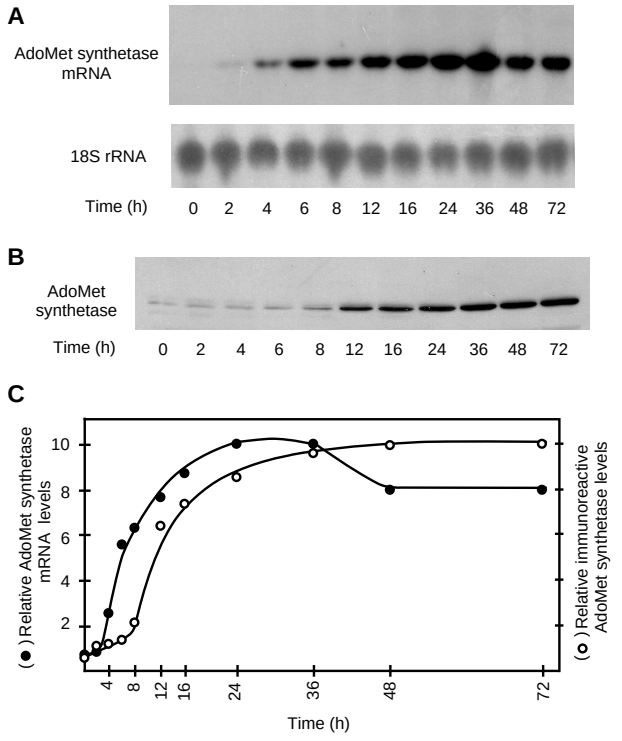

FIG. 2. Time course of hepatic AdoMet synthetase mRNA and immunoreactive protein induction in rat hepatoma H35 cells in response to triamcinolone. H35 cells were treated with 100 nM triamcinolone, and total RNA was extracted at the indicated times. A, Northern blot analyses, performed with 30 μg total RNA. Filters were hybridized to a ^{32}P-labeled rat hepatic AdoMet synthetase or to a rat 18S ribosomal RNA probe. B, For Western blot, equal amounts of cytosolic proteins (30 μg) were fractionated by 10% SDS-PAGE and subjected to immunoblot analysis with an antibody raised against the liver-specific AdoMet synthetase (dilution, 1:10,000). Densitometric quantitation is shown below. Immunoreactive protein and mRNA levels are expressed relative to the maximal value, which was taken as 10.

Effects of cycloheximide and actinomycin D on the triamcinolone-dependent increase in AdoMet synthetase mRNA

To determine whether the effect of triamcinolone on AdoMet synthetase transcripts was dependent on protein synthesis, cultures of H35 cells were treated with 100 nM triamcinolone in the presence or absence of 10 μg/ml cycloheximide. After 8 h of treatment, the induction of AdoMet synthetase mRNA was not inhibited by cycloheximide (Fig. 6), indicating that on-going protein synthesis is not required for triamcinolone-dependent AdoMet synthetase mRNA induction. This result suggests that preexisting glucocorticoid receptors mediated the triamcinolone up-regulation of AdoMet synthetase mRNA. On the other hand, to determine whether the glucocorticoid effect on hepatic AdoMet synthetase expression was at the transcriptional level, cultures of H35 cells were treated for 8 h with 100 nM triamcinolone in the presence of the transcriptional inhibitor actinomycin D at a concentration of 5 μg/ml. As shown in Fig. 6, this treatment prevented stimulation by triamcinolone, suggesting that AdoMet synthetase mRNA induction by glucocorticoids is transcriptionally regulated.

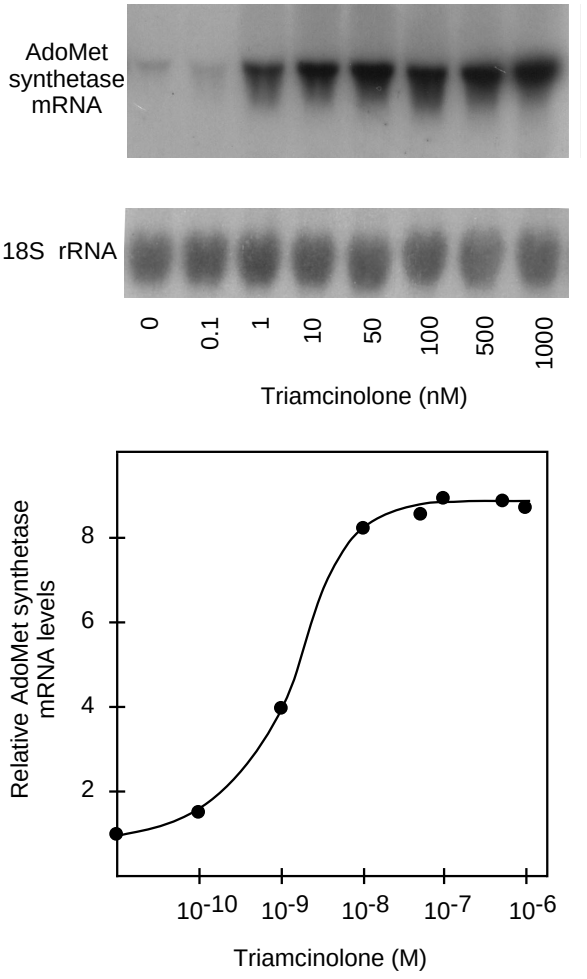

FIG. 3. Triamcinolone up-regulates AdoMet synthetase mRNA in a dose-dependent manner. H35 cells were incubated for 16 h with increasing concentrations of triamcinolone. Northern blot was performed as detailed in *Materials and Methods*. A semilogarithmic plot showing normalized hepatic AdoMet synthetase mRNA levels *vs.* hormone concentration is depicted below.

Transcriptional regulation of the hepatic AdoMet synthetase gene by triamcinolone

To determine more directly whether glucocorticoids control the transcription of the hepatic AdoMet synthetase gene, nuclear run-on assays were performed on nuclei from H35 cells treated with 100 nM triamcinolone for 1 or 6 h. The behavior of the gene coding for cytosolic PEPCK was simultaneously tested as an internal standard of enzymes transcriptionally modulated by glucocorticoids in hepatoma cells (37). As illustrated in Fig. 7A, a significant increase in the transcriptional activities of both PEPCK and hepatic AdoMet synthetase genes was achieved after triamcinolone stimulation. Quantification of the data from three independent experiments revealed that the relative rate of transcription of the hepatic AdoMet synthetase gene, after normalization to GAPDH transcription rate, was increased about 2.8-fold 1 h after triamcinolone addition and 3.2 fold at 6 h.

To further characterize the effects of triamcinolone on transcription of the AdoMet synthetase gene, H35 cells were transiently transfected with a construct containing a 1.4-kb fragment of the rat liver-specific AdoMet synthetase promoter fused to the luciferase reporter gene. Treatment of transfected cells with 100 nM triamcinolome for 18 h produced a 3-fold increase in luciferase activity (Fig. 7B), which agrees with the results of the run-on analysis.

Effects of triamcinolone on the turnover of hepatic AdoMet synthetase mRNA

We also evaluated whether glucocorticoids alter the turnover of hepatic AdoMet synthetase mRNA. The decay of AdoMet synthetase mRNA was followed in H35 cells that had been treated with 100 nM triamcinolone. Sixteen hours after triamcinolone addition, the hormone was either maintained or removed, and the cells were incubated for additional times in the presence or absence of actinomycin D (Fig. 8). When triamcinolone was withdrawn and the cells were cultured in the absence of the transcription inhibitor, the mRNA decayed with a first order kinetics, exhibiting a half-life of about 7 h. The rate of decay of the mRNA was much slower after actinomycin D addition either in the continued presence or after withdrawal of triamcinolone.

Discussion

An important physiological action of glucocorticoids is the control of the expression of hepatic genes encoding regulatory enzymes of intermediary metabolism. Some of these enzymes are involved in hepatic amino acid metabolism, such as tyrosine aminotransferase (38) and the urea cycle enzymes (39). In the present report, we show that these hormones strictly regulate the liver-specific AdoMet synthetase, the enzyme that catalyzes the preferred pathway for methionine breakdown. Previous studies had shown that adrenalectomy reduces AdoMet synthetase activity in rat liver, and cortisone administration can effectively prevent this effect (20). Our results reveal that after adrenalectomy and triamcinolone replacement, changes in the specific activity of the enzyme correlate with changes in immunoreactive protein and mRNA contents, therefore suggesting that *in vivo* regulation of hepatic AdoMet synthetase by glucocorticoids is mediated at the mRNA level. A direct and specific action of glucocorticoids on the enzyme gene expression has been demonstrated by using two cell culture systems, rat hepatoma H35 cells and primary cultures of adult rat hepatocytes. It is noteworthy that after triamcinolone stimulation, the response and kinetics of hepatic AdoMet synthetase mRNA expression were similar in both systems despite the fact that basal levels of the mRNA were low in the hepatoma cells. This interesting finding reveals that the machinery involved in glucocorticoid-mediated regulation of AdoMet synthetase gene expression is conserved in a dedifferentiated cell line such as rat hepatoma H35. Also consistent with the results derived from *in vivo* studies, the increase in mRNA content was paralleled by increased levels of immunoreactive AdoMet synthetase in cultured cells. Although the induction profiles of hepatic AdoMet synthetase mRNA and protein were almost identical, a pronounced delay in the accumulation of protein with respect to the mRNA was detected. This effect has been evidenced for a variety of en-

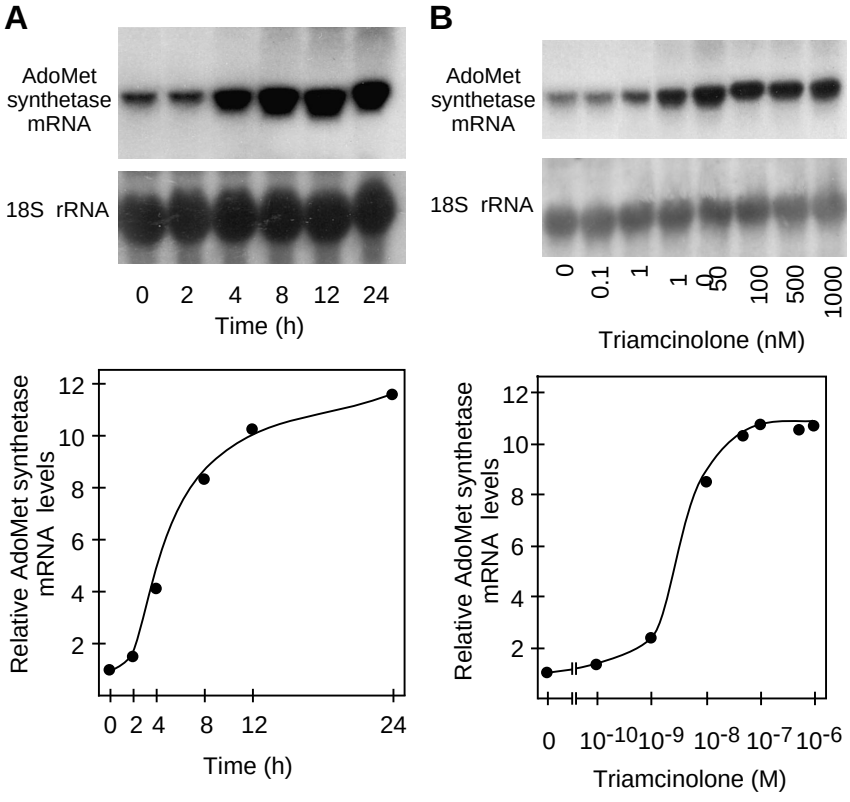

FIG. 4. Up-regulation of hepatic AdoMet synthetase mRNA by triamcinolone in primary cultures of adult rat hepatocytes. A, Time course of hepatic AdoMet synthetase mRNA induction in response to triamcinolone. Hepatocytes were isolated following the classical collagenase perfusion method, cultured as detailed in *Materials and Methods*, and treated with 100 nM triamcinolone. RNA was extracted at the indicated times. Northern blot analysis was performed with 20 μg total RNA. Filters were hybridized to a ^{32}P-labeled rat hepatic AdoMet synthetase or to 18S ribosomal RNA probe. The corrected densitometric quantitation is shown below. The mRNA levels are expressed relative to the value at time zero, which was taken as 1. B, Dose-dependent effect of triamcinolone on AdoMet synthetase mRNA. Primary cultures of rat hepatocytes were incubated for 12 h with increasing concentrations of triamcinolone. A semilogarithmic plot showing normalized hepatic AdoMet synthetase mRNA levels *vs.* hormone concentration is shown below. Each autoradiogram presented is representative of three independent experiments.

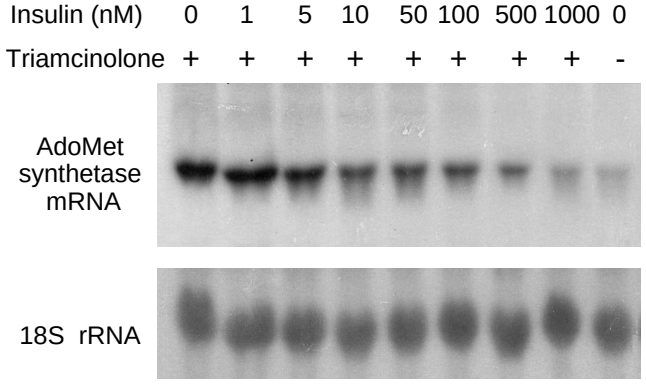

FIG. 5. Inhibition by insulin of the triamcinolone-induced increase of AdoMet synthetase mRNA levels. H35 cells were incubated for 24 h with 100 nM triamcinolone and the indicated concentrations of insulin. Hormones were added alone or simultaneously as indicated. Northern analysis was performed with 30 μg total RNA.

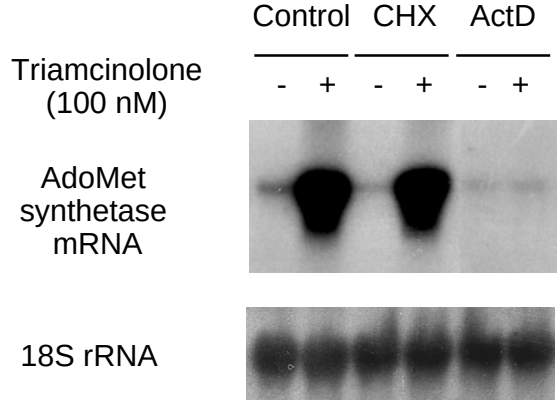

FIG. 6. Actinomycin and cycloheximide effects on hepatic AdoMet synthetase inducibility by triamcinolone. H35 hepatoma cells were incubated for 8 h in the presence of 100 nM triamcinolone or vehicle. When indicated, either actinomycin at 5 μg/ml or cycloheximide at 10 μg/ml was added to the culture medium simultaneously with the hormone. Northern blot analysis was performed with 30 μg total RNA. Hybridization with 18S ribosomal RNA probe is shown below.

zymes (40) and could be explained by the time required for AdoMet synthetase translation from mRNA to protein.

The finding that glucocorticoid-mediated up-regulation of hepatic AdoMet synthetase mRNA expression is antagonized by insulin might be related to the fact that the action of glucocorticoids in the regulation of carbohydrate, protein, and lipid metabolism is commonly opposed by insulin. In this context, a negative effect of insulin on glucocorticoid-induced mRNA levels has been reported for several proteins involved in hepatic metabolism, such as tyrosine aminotransferase (41), PEPCK (37), or IGF-binding protein-1 (42). The mechanisms by which insulin antagonizes glucocorticoid regulation of gene expression are still poorly understood. Recent reports have provided evidence that in hepatic cells, insulin may act indirectly by interfering with transcription factors necessary for the glucocorticoid response (43–45). Other possible mechanisms could be related to the ability of the insulin-degrading enzyme to interact with the glucocorticoid receptor, which has been suggested to potentially couple insulin and steroid hormone signaling pathways (46).

Run-on transcription assays with isolated nuclei have provided direct evidence for a transcriptional activation of the hepatic AdoMet synthetase gene by glucocorticoids. In keep-

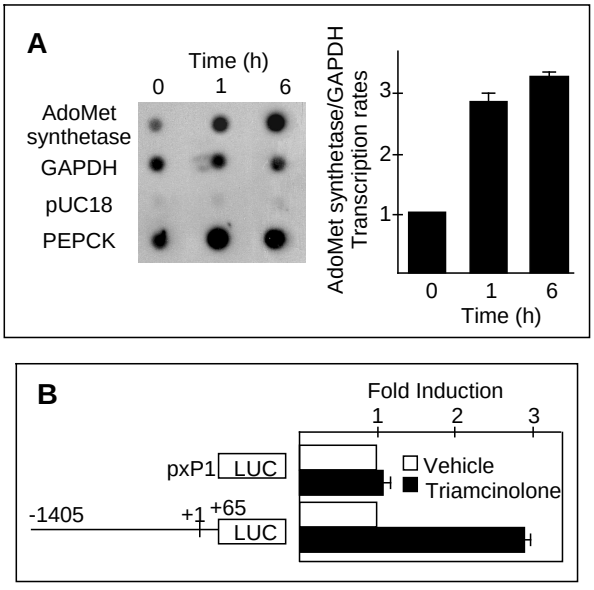

FIG. 7. Effects of glucocorticoids on rat liver AdoMet synthetase gene transcription. A, Nuclear run-on analysis of the hepatic AdoMet synthetase transcription rate after triamcinolone treatment. The transcription run-on assay was carried out with nuclei isolated from H35 cells untreated or treated for 1 and 6 h with 100 nM triamcinolone. PEPCK and GAPDH transcription rates were tested simultaneously. The histogram shows a quantification of the AdoMet synthetase transcription rate normalized to that one of GAPDH. B, Effects of glucocorticoids on rat liver AdoMet synthetase gene promoter. Fifteen micrograms of AdoMet synthetase-luciferase fusion construct or the vector alone were transfected into H35 cells in the presence of 5 μg of an internal standard, Rous sarcoma virus-β-galactosidase. Cells were incubated with or without 100 nM triamcinolone 24 h before reporter assays. Luciferase activities were normalized to β-galactosidase expression. Values are expressed as the mean $\pm$ SEM of three separate experiments.

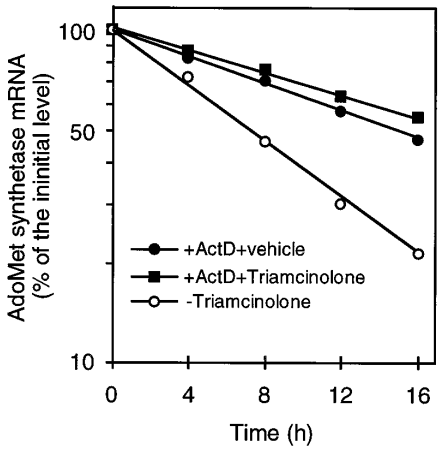

FIG. 8. Time course of hepatic AdoMet synthetase mRNA decay after triamcinolone removal or after the addition of actinomycin D. H35 rat hepatoma cells were cultured in the presence of 100 nM triamcinolone for 16 h before deinduction. Triamcinolone was withdrawn by washing the monolayer three times with PBS, and cells were incubated for additional times. In another set of experiments, actinomycin D (5 μg/ml) was added after hormone induction, and cells were then incubated in the presence or absence of triamcinolone. Cells were harvested at the indicated times, and total RNA was extracted. Data are plotted as a percentage of the AdoMet synthetase mRNA content before deinduction. A representative result from three independent experiments is shown.

ing with this, the activity of the promoter was also induced to a comparable level upon hormone stimulation, as deduced from the transient transfection assays of reporter plasmids containing 1.4 kb of the 5′-flanking region of the rat liver AdoMet synthetase gene. Therefore, it can be concluded that the functional sequence elements conferring glucocorticoid inducibility are present in this promoter region. In this regard, it is interesting to note that it contains four putative GREs (Alvarez, L., G. Sanchez-Góngora, J. Mingorance, B. Gil, M. A. Pajares, manuscript in preparation; database accession number X80270). Further studies are required to verify whether these elements, either alone or in a combined fashion, are mediating the glucocorticoid responsiveness of the rat hepatic AdoMet synthetase gene.

Although glucocorticoids are primarily considered to function by changing the rate of transcription of the target genes (47, 48), they have been reported to also modulate specific mRNA levels at the posttranscriptional level, including alteration of mRNA stability (49, 50) and stimulation of transport from the nucleus to cytoplasm (51). Such combined actions, which can lead to more rapid and more marked shifts in the expression of particular genes, also appear to apply to the regulation of rat hepatic AdoMet synthetase, as the 3-fold enhancement of the gene transcription rate does not quantitatively match the 14-fold increase in mRNA content. Thus, although the action of glucocorticoids on AdoMet synthetase gene expression is clearly a direct one, a second factor may be required to sustain the hormonal effect. This is consistent with the results derived from the mRNA turnover studies. In contrast with the disappearance of the AdoMet synthetase mRNA after removal of triamcinolone, its rate of decay after addition of the transcriptional inhibitor actinomycin D was much slower, raising the possibility that a short lived factor may be involved in the turnover process. A similar suggestion has been derived from a number of studies of various mRNAs in different cell types (22, 52–54).

AdoMet synthetase plays a crucial role in hepatic metabolism, processing most of the methionine taken up from the diet and providing key metabolites for the cell. Therefore, the glucocorticoid-mediated regulation of this enzyme could be a factor of major physiological significance, especially in those situations when the levels of these hormones are high, such as in stress conditions or during the peak of the glucocorticoid circadian rhythm. On the other hand, it should be mentioned that glucocorticoids are used as therapeutic agents in alcoholic liver cirrhosis. Interestingly, AdoMet synthetase activity has been reported to be seriously reduced in such a liver disorder (9, 10). Furthermore, it has been shown that long term consumption of ethanol in baboons leads to a depletion of AdoMet levels, and administration of this metabolite attenuates ethanol-induced liver damage (55). Therefore, it is tempting to speculate that at least some of the beneficial effects of glucocorticoids in liver cirrhosis could be due to a direct stimulation of AdoMet synthetase, which, in turn, would increase the availability of AdoMet. In keeping with this, the data provided here could also offer new insights into the mechanisms by which glucocorticoids exert therapeutic effects in alcohol-induced liver damage.

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